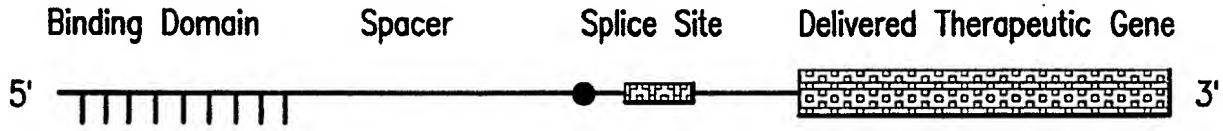

Binding Domain
Spacer
Splice Site
Delivered Therapeutic Gene
5'
3'

FIG.1A

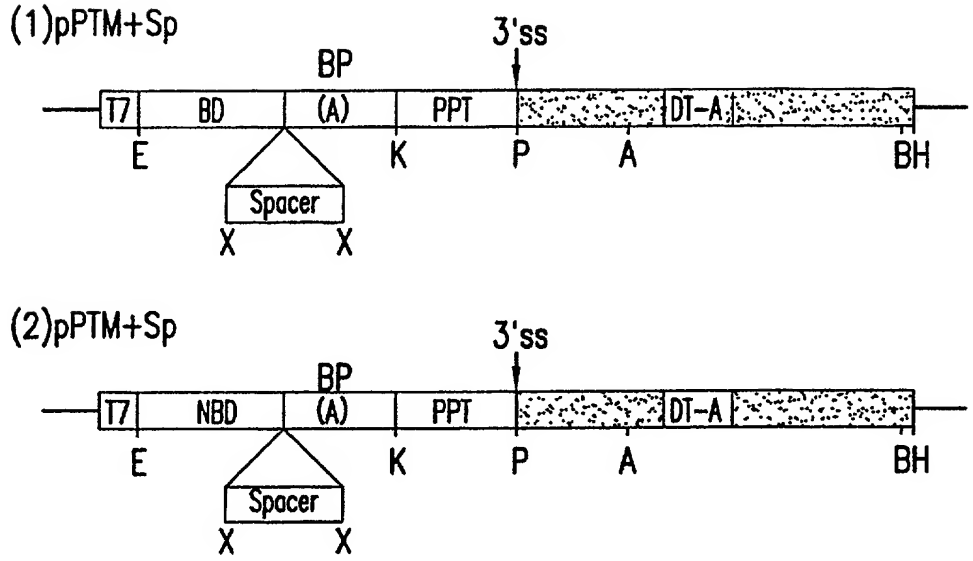


FIG.1B

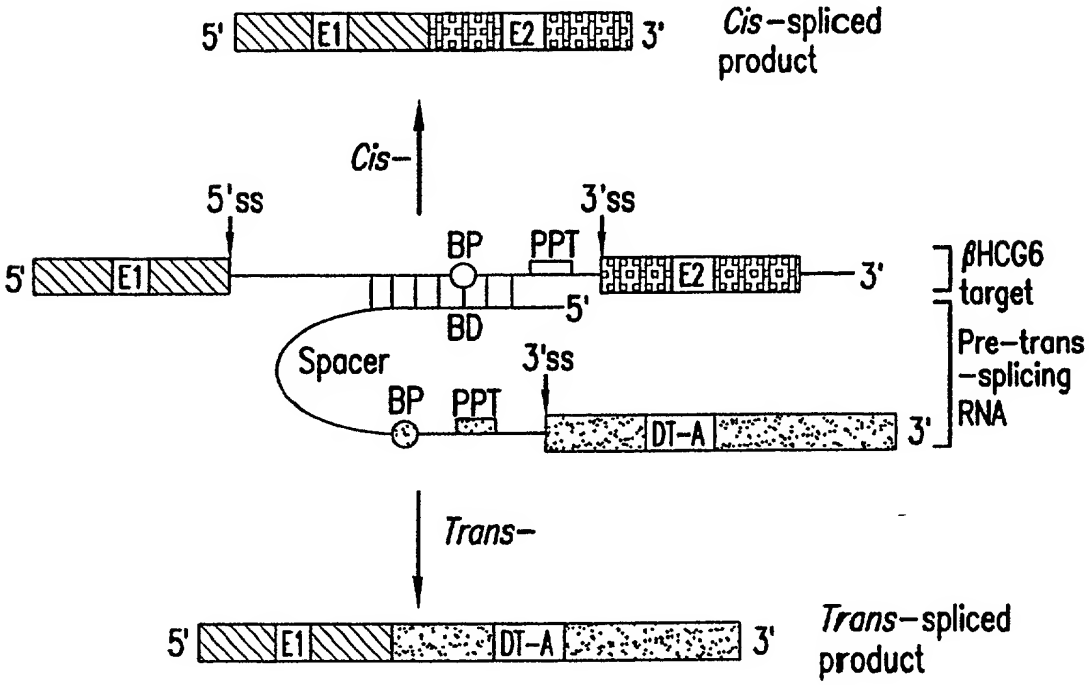


FIG.1C

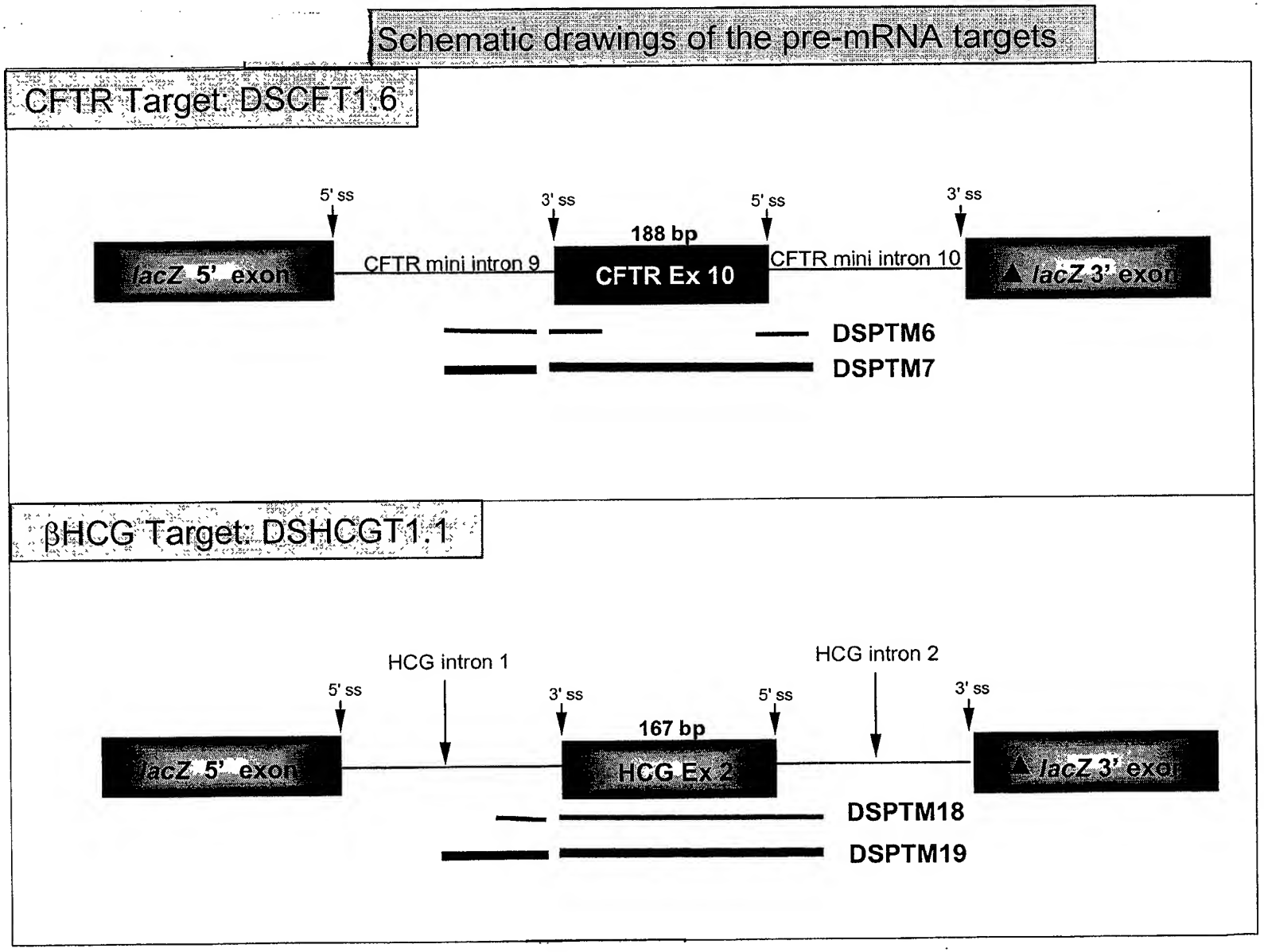

Schematic drawings of the pre-mRNA targets
CFTR Target: DSCFT1.6
5' ss
3' ss
5' ss
3' ss
188 bp
lacZ 5' exon
CFTR mini intron 9
CFTR Ex 10
CFTR mini intron 10
lacZ 3' exon
DSPTM6
DSPTM7
βHCG Target: DSHCGT1.1
HCG intron 1
HCG intron 2
5' ss
3' ss
5' ss
3' ss
167 bp
lacZ 5' exon
HCG Ex 2
lacZ 3' exon
DSPTM18
DSPTM19

Figure 2

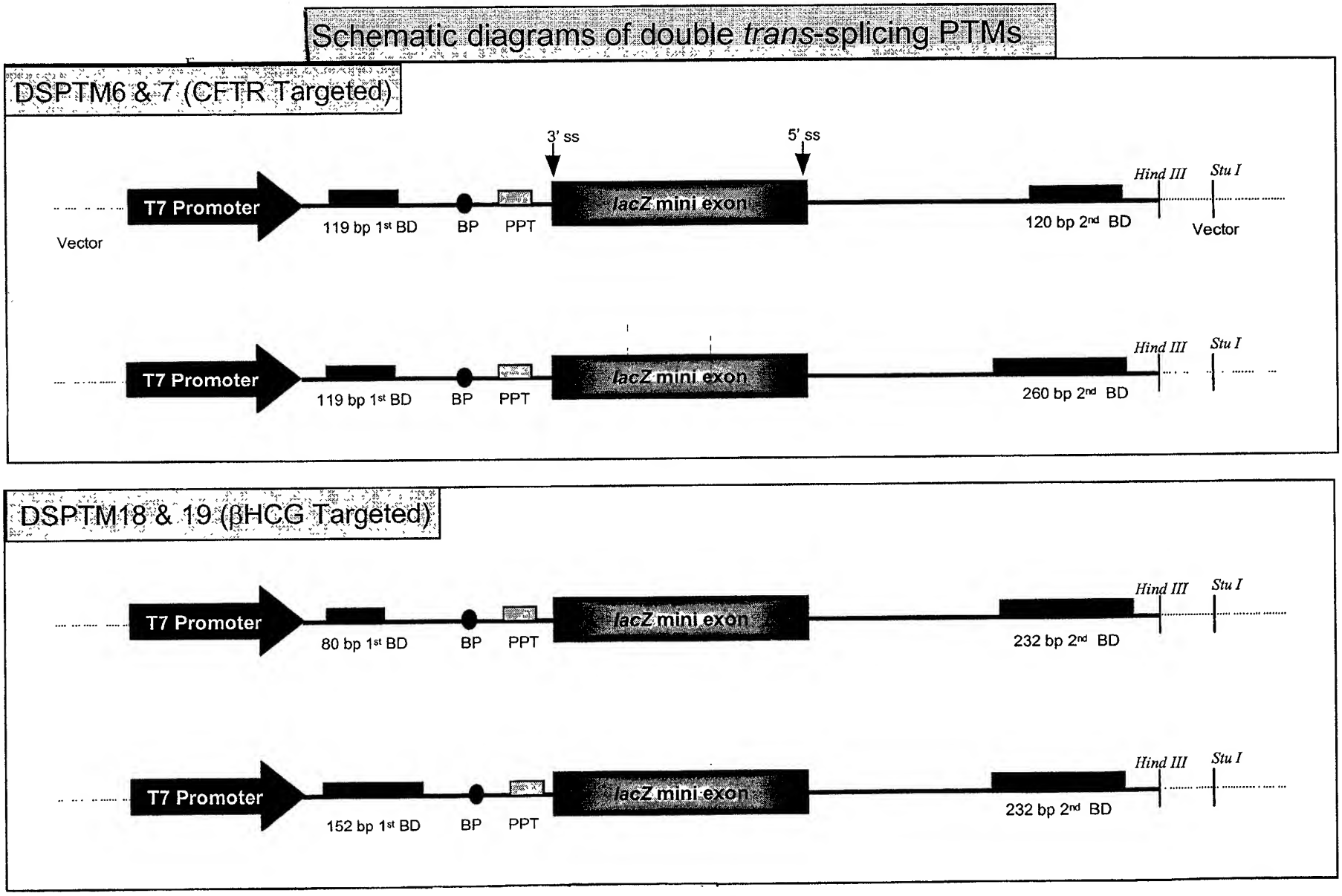
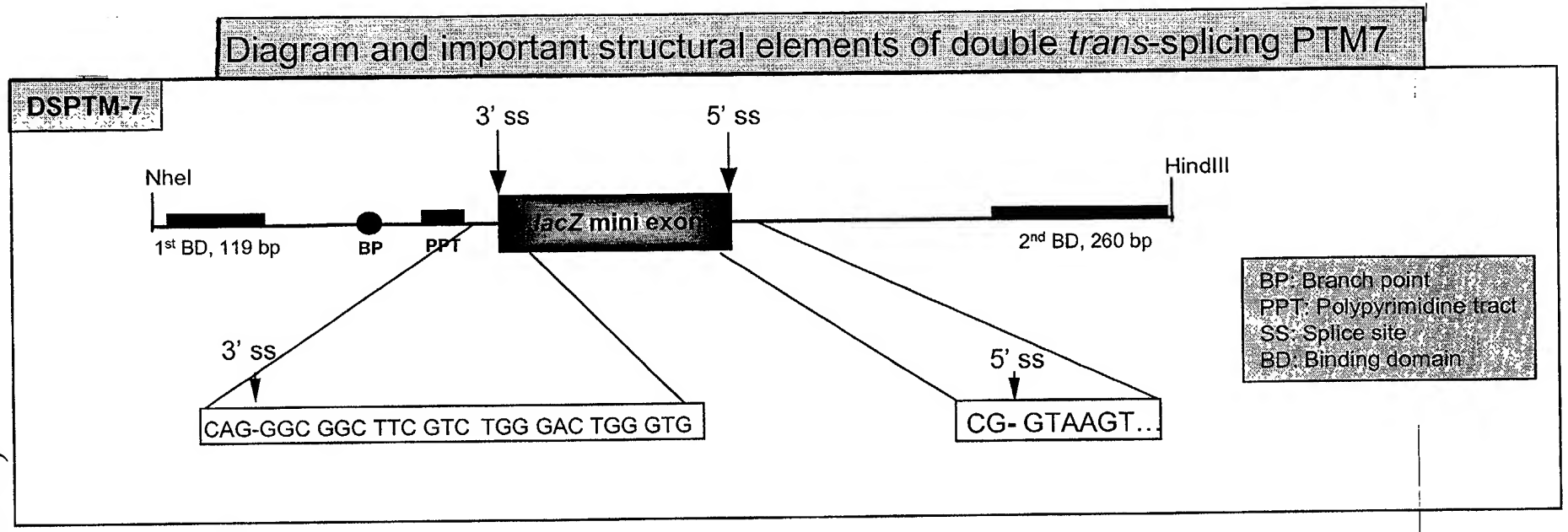

Schematic diagrams of double trans-splicing PTMs
DSPTM6 & 7 (CFTR Targeted)
3' ss
5' ss
Hind III
Stu I
T7 Promoter
lacZ mini exon
Vector
119 bp 1st BD
BP
PPT
120 bp 2nd BD
Vector
T7 Promoter
119 bp 1st BD
BP
PPT
lacZ mini exon
260 bp 2nd BD
DSPTM18 & 19 (βHCG Targeted)
T7 Promoter
80 bp 1st BD
BP
PPT
lacZ mini exon
232 bp 2nd BD
T7 Promoter
152 bp 1st BD
BP
PPT
lacZ mini exon
232 bp 2nd BD

Figure 3



1st BD (119 bp) : GATTCACTTGCTCCAATTATCATCCTAAGCAGAAGTGTATATTCTTATTTGTAAAGATTCTATTAACTCATTTGATTCAAAATA
TTTAAAATACTTCCTGTTTCATACTCTGCTATGCAC

Spacer sequences: AACATTATTATAACGTTGCTCGAA

BP, PPT and acceptor splice site: TACTAAC T *GGTACC* TCTTCTTTTTTTTTT *GATATC* CTGCAG GGC GGC TTC GTC TGG GAC TGG

BP PPT 3' ss *lacZ* mini exon

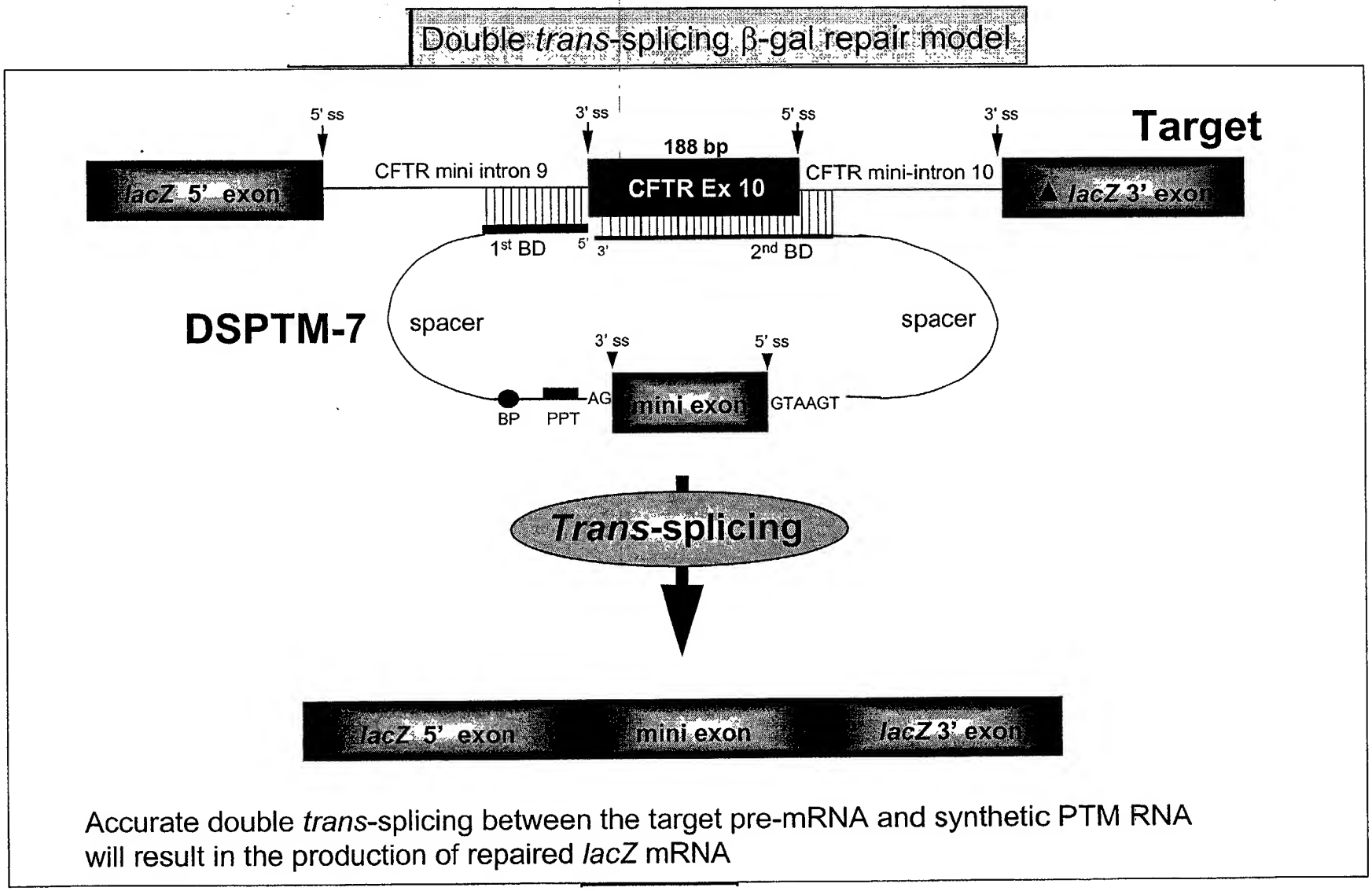
5' donor site and 2nd spacer sequence: TGA ACG *GTAAGT* GTTATCACCGATATGTGTCTAACCTGATTCGGGCCTTCGATACGCTAA
GATCCACCGG

lacZ mini exon 5' ss

2nd BD (260 bp): TCAAAAAGTTTTCACATAATTTCTTACCTCTTCTT*GAATTC*ATGCTTTGATGACGCTTCTGTATCTATATTCATCATTGGAA
ACACCAATGATTTTTCTTTAATGGTGCCTGGCATAATCCTGGAAAACTGATAACACAATGAAATTCTTCCACTGTGCTTAA
AAAAACCCTCT*GAATTC*TCCATTTCTCCCATAATCATCATTACAACTGAACTCTGGAAATAAAACCCATCATTATTAACTCA
TTATCAAATCACGC

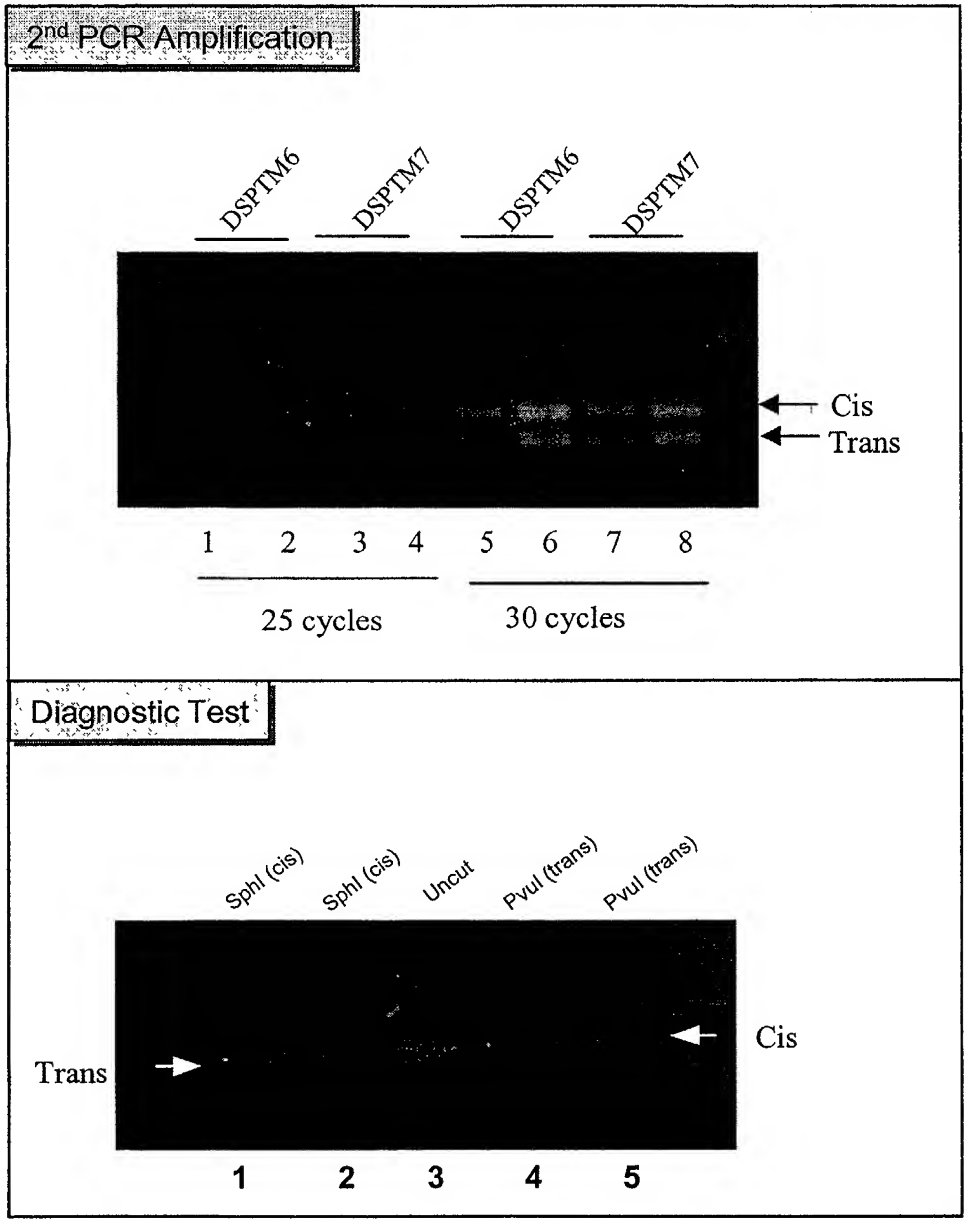
Figure 4

Figure 5



Accurate double *trans*-splicing between the target pre-mRNA and synthetic PTM RNA will result in the production of repaired *lacZ* mRNA

Proof-of-principle of SMaRT using synthetic double splicing PTM RNA in 293T cells



DSPTM6 and 7 (CFTR targeted)

Methods

Transfect 293T cells with DSPTM6 and DSPTM7 *in vitro* transcribed, gel purified RNA (2.5-5.0 µg)

Isolate total RNA, cDNA synthesis (Lac6R), PCR amplification (20 cycls, KI-1F + Lac6R), digest with *Sph* I + *Dde* I (*cis*-specific) at 37°C/ON

Purify double *trans*-spliced product using Biotin-Lac21R probe

PCR amplify the captured *trans*-spliced product (KI-2F+Lac6R). Expected products: *cis*- 260bp; *trans*- 220 bp.

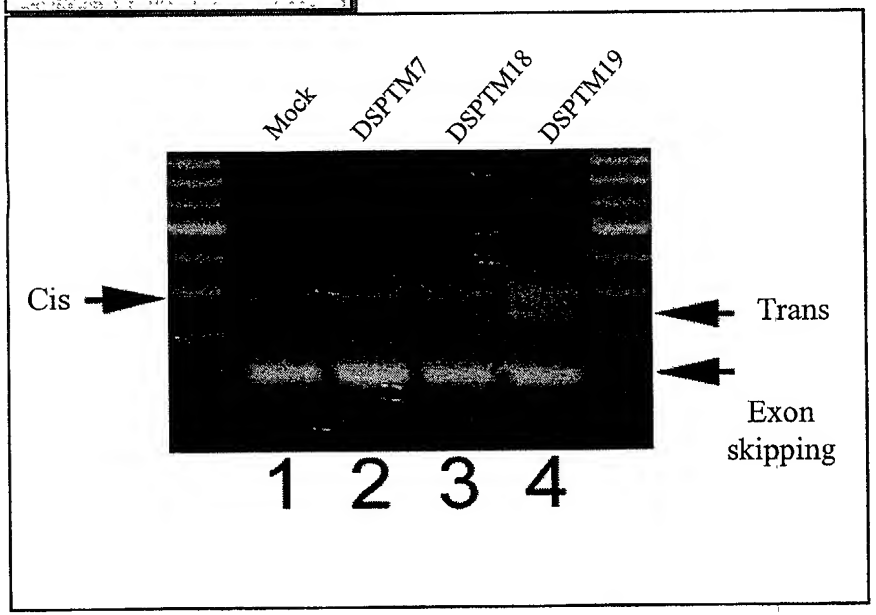
Diagnostic test: Digest PCR product with *Pvu* I (*trans*-specific) and with *Sph* I (*cis*-specific) at 37°C for 2-3 hr

Sequence to confirm the accuracy of double *trans*-splicing

Figure 6A

Proof-of-principle of SMaRT using synthetic double splicing PTM RNA in stable cells

2nd PCR Amplification



DSPTM18 and 19 (HCG targeted)

Methods

Transfect DSHCGT1.1 stable cells with DSPTM7, DSPTM18 and DSPTM19 *in vitro* transcribed, gel purified RNA (2.5-5.0 μg)

Isolate total RNA, cDNA synthesis (Lac6R), PCR amplification (20 cycls, KI-1F + Lac6R), digest with *Sph* I + *Dde* I (*cis*-specific) at 37°C/ON

Purify double *trans*-spliced product using Biotin-Lac21R probe

PCR amplify the captured *trans*-spliced product (KI-2F + Lac6R). Expected products: *cis*- 260bp; *trans*- 220 bp

Sequence to confirm the accuracy of double *trans*-splicing

Figure 6B

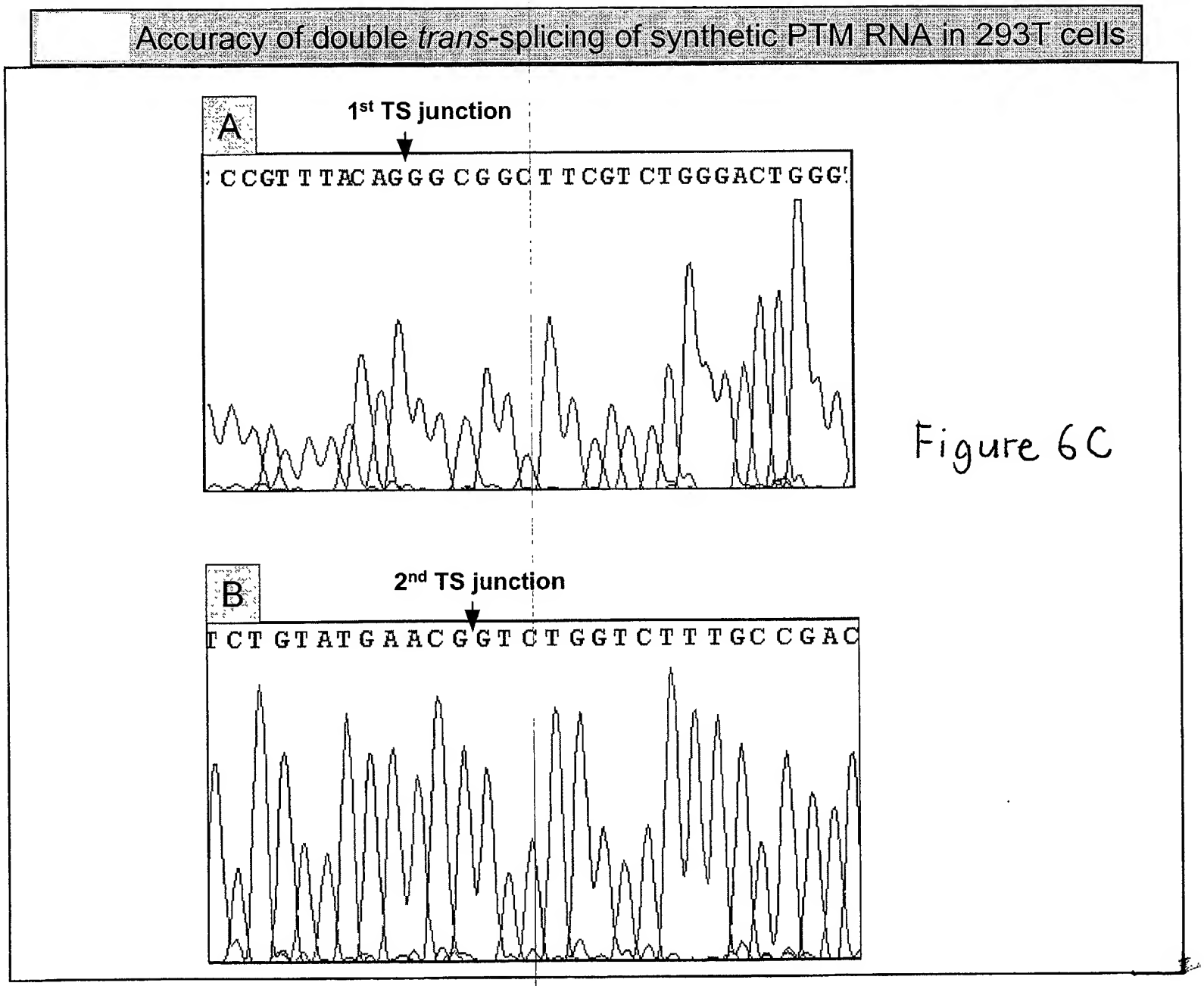
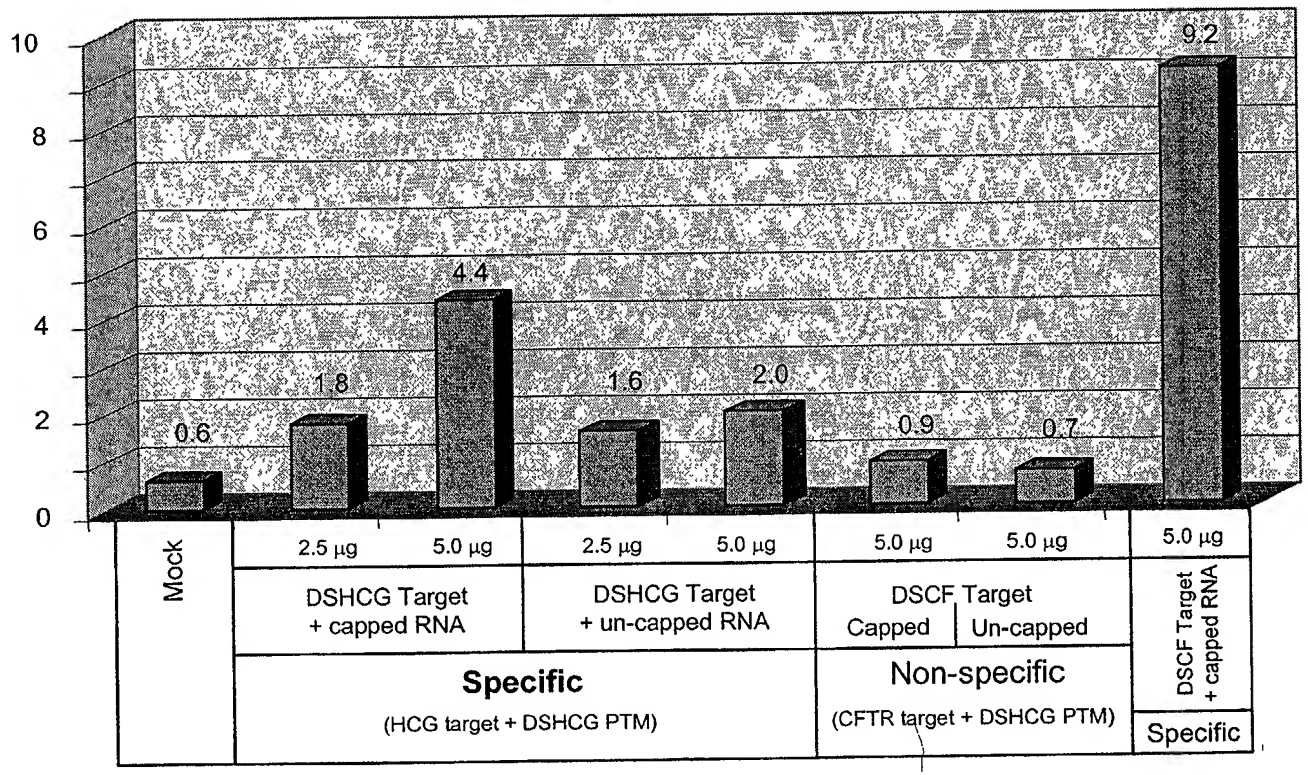

Accuracy of double *trans*-splicing of synthetic PTM RNA in 293T cells
A
1st TS junction
: C C G T T T A C A G G G C G G C T T C G T C T G G G A C T G G G
B
2nd TS junction
T C T G T A T G A A C G G T C T G G T C T T T G C C G A C

Figure 6C

Restoration of β-gal function through RNA transfection in 293T cells
(Proof-of-concept for SMaRT RNA Therapeutics!!)
Synthetic RNA, Double *trans*-splicing



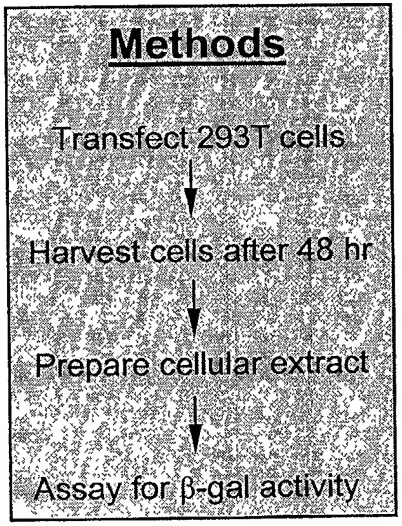


Figure 7